INTERRELATIONSHIPS IN THE SOIL MICROBIAL BIOCENOSIS UNDER SPRUCE STAND AFFECTED BY SO_2 IMMISSIONS

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Abstract
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Simple and partial correlation coefficients were calculated for the determination of the relations among immission effects, nutrient content, microbe concentrations and their activities in the fermentative horizon A_{02} under five spruce stands exposed to different levels of SO_2 pollution. For the explanation of the adverse effect of immissions on soil activities the following mechanisms were proposed: a) increased H^+ concentration influences the physiology of microbes, changes their enzyme production, the released enzymes are distant from optimum pH, b) immissions act on the accumulation of carbonaceous substances associated with changes in C:N ratio, c) toxic sulphur compounds break the structural, and thereby also functional integrity of microbes; they alter their enzymic outfit and inhibit the liberated enzymes.

Even despite the wide range of factors under study, the series of observations used in the trials (Langkramer, Lettl, 1982) does not give sufficiently solid basis for full explanation of the effect of SO_2 immissions on the microbial biocenosis of soil.

Introduction

The adverse effect of the SO_2 industrial immissions is beyond any doubt now. It has been demonstrated by ecological studies directly in the polluted terrain (Langkramer, 1967, 1974, 1975; Mrkva, Grunda, 1969; Langkramer, Lettl, 1981, 1982) and verified by soil fumigation with SO_2 (Labeda, Alexander, 1978; Grant et al., 1979 a, b; Dodd, Lauenroth, 1981). The effect on soil is a first-rate problem since the reduced activity of microorganisms results in a lower soil fertility.

The mechanism of the action of SO_2 immissions is most frequently associated with the acidification of the soil (Jonsson, 1976; Bryant et al., 1979), but it has been found that this is not the only factor (Wodzinski, Alexander, 1978). Hence consideration is given to the toxicity of SO_3^{2-} /HSO_3^- as solubilization products of SO_2 (Wodzinski et al., 1978; Grant et al., 1979a). However, the experimental use of the former (Abrahamsen et al., 1976; Bååth et al., 1979) and the latter factor (Hill, 1971; Lettl, 1980, 1981) gave just partial simulation of the effects of immissions. This is probably due to the fact that long term exposure to acid rains

and to SO_2 pollution under natural conditions causes not only acidification and a decrease in exchange pH (Lochman et al., 1981) but also soil changes manifesting themselves as a loss in nutrients, cation outwash, mobilization of Al^{3+} (McFee, 1980; Lochman, 1981) and an increase in the SO_4^{2-} content (Pelíšek, 1976; Lettl et al., 1981) and other intermediate products of the sulphur cycle (Wainwright, 1978a). Hence in fact the adverse effect of SO_2 immissions has been explained just partially.

This paper is aimed at contributing, from the ecological position, to the explanation of the adverse effects of SO_2 immissions on the microbial biocenosis of soil. The interrelationships among nutrient substrates, microorganisms and their activities were analyzed as influenced by the SO_2 immissions. The analysis is based on a nine-year series of observations of the effect of immissions on the soil microflora under a spruce stand (Langkramer, Lettl, 1981, 1982). The paper studies only the conditions in the fermentative horizon A_{02} where the effect of immisions is most pronounced and where microbial activities are at the highest level.

Material and Methods

Five test plots with spruce stands were chosen in the Slavkov Forest in Western Bohemia. The plots are ordered by the average content of SO_4^{2-} in the fermentative horizon A_{02}. The range is from relatively "pure" plots I and II (without signs of stand damage) through slightly polluted plot III (damage grade I) up to plots IV and V heavily polluted with the SO_2 immissions (stand damage grade II). Besides SO_2, the last plot (V) is also polluted with fly ash from the nearby power station. Detailed characteristics were given in another paper (Langkramer, 1975).

The concentrations of aerobic and ammonifying bacteria, thiobacilli and microfungi were studied in the test plots since 1971—1974 to 1978—1979. As to the soil biochemical activities, the following ones were studied: basal and potential respiration, ammonification, nitrification, $S_2O_3^{2-}$ and S^0 oxidation. The studied soil characteristics included moisture, pH(H_2O), pH(KCl), organic carbon content (C_{ox}), total nitrogen content (N_{total}), C:N ratio, content of NH_4^+—N, NO_3^-—N and SO_4^{2-}. Detail data are given in another papers (Langkramer, Lettl, 1981, 1982).

Simple and partial correlation coefficients were calculated from the results of the analyses of individual samplings for the studied period, for all the five plots at the same time. The significance of correlation coefficients was tested at the level of $P = 0.9$ ($\alpha = 0.1$) (Likeš, Laga, 1978).

Results

The arithmetic means of the characteristics studied in the five test plots are summarized in Tab. 1. In view of the different altitudes above sea level it is advisable to compare plots II, IV and V (about 500 m above sea) separately from plots I and III (about 800 m above sea).

The simple correlations ($P = 0.9$) for all the five plots at the same time are denoted in Tab. 2, partial correlations in Tab. 3. The causal relations ensuing from this are tentatively shown in Figures 1 and 2.

Table 1. Mean characteristics of five research plots

Research plot	I	II	III	IV	V
Elevation above sea level in m	870	540	835	490	620
Damage degree of forest stand	0	0	I	II	II
Moisture [%]	56.5	50.9	55.0	50.4	52.3
pH_{H_2O}	3.70	3.82	3.56	3.86	3.78
pH_{KCl}	2.97	3.19	2.82	3.35	3.11
C_{ox} [%]	38.9	38.7	39.3	34.0	40.0
N_{total} [%]	1.56	1.38	1.55	1.37	1.46
C:N	25.6	28.4	25.5	24.7	24.2
NH_4^+—N [ppm]	131.9	104.5	109.2	73.4	233.2
NO_3^-—N [ppm]	12.9	4.9	4.0	20.2	9.0
SO_4^{2-} [ppm]	203	237	249	309	383
Scope of microbial biocenosis (in thousands per g oven dry soil)					
Aerobic bacteria	888	3683	730	2537	1450
Ammonifying bacteria	1198	4255	1068	4554	1501
Thiobacilli	109	63.6	10.6	398	283
Microfungi	488	780	736	1110	694
Soil activities [in $mg.kg^{-1}.t^{-1}$]					
Respiration	2223	2360	1729	1385	1392
Ammonification	115	147	89.8	79.1	49.2
Nitrification	3.72	−2.94	6.49	12.6	−0.43
Oxidation of $S_2O_3^{2-}$	6720	6148	6333	4686	4918
Oxidation of S^0	7002	6811	9751	7579	10 040

Discussion

The SO_2 pollution increases the concentration of hydrogen ions and exchangeable H^+ ions in the soil (McFee, 1980; Lochman et al., 1981) and the concentration of SO_4^{2-} (Pelíšek, 1976; Lettl et al., 1981). These three factors which are positively correlated with one another are therefore considered as the primary manifestation of SO_2 pollution in the soil milieu.

In our results the concentrations of $H^+(H_2O)$ and $H^+(KCl)$ are negatively correlated with the concentrations of aerobic and ammonifying bacteria and with the biochemical activities of the soil; therefore the actual and exchangeable soil pH value seems to be a decisive factor. This view is also supported by experimental material: the artificial acidification of the soil reduced the numbers of bacteria and active mycelium and inhibited respiration (Bååth et al., 1979; Bryant et al., 1979; Grant et al., 1979b) and nitrification (Abrahamsen et al., 1976). Indirect evidence is provided by the fact that an adjustement of soil pH by liming restored the soil biological activity (Król et al., 1972) including an increase in S^0 oxidation (Attoe,

Table 2. Simple correlations (+) positive and (−) negative

	SO_4^{2-}	$H^+_{H_2O}$	H^+_{KCl}	Moisture	C_{ox}	N_{total}	C:N	NH_4^+—N	NO_3^-—N	Aerobic bacteria	Ammonifying bacteria	Thiobacilli	Microfungi	Respiration	Ammonification	Nitrification	Oxidation of $S_2O_3^{2-}$	Oxidation of S°
SO_4^{2-}		+			−										−		−	
$H^+_{H_2O}$	+									−	−			−	−			
H^+_{KCl}					−	−			+					−		+	−	
Moisture					+	+					+	+		+	+		+	+
C_{ox}	−		−	+		+	+		−			+		+	+	−	+	
N_{total}			−	+	+		−		−			+		+			+	+
C:N					+	−									+			−
NH_4^+—N																		
NO_3^-—N			+		−	−				−				−	−	+		
Aerobic bacteria		−							−		+			+				
Ammonifying bacteria		−		+						+				+	+			
Thiobacilli				+	+	+							+	+				
Microfungi												+						
Respiration		−	−	+	+	+			−	+	+	+			+	−	+	
Ammonification	−	−		+	+		+		−		+			+		+	+	
Nitrification			+		−				+					−	+			
Oxidation of $S_2O_3^{2-}$	−		−	+	+	+								+	+			+
Oxidation of S°				+		+	−					+					+	

Olson, 1966; Lettl et al., 1981). Hence it can be considered as demonstrated that an increase in H^+ concentration is a primary adverse factor. Acidification may affect organisms at physiological level; it may alter the quantity and quality of produced enzymes. The produced free enzymes may be inhibited by a shift of pH outside the zone of activity. A reduction in soil activity is the result of these effects. These adverse effects of H^+ concentration are tentatively represented by chart A in Fig. 3.

The positive correlations between bacteria and their activities testify just partly to the causality of these organisms for the given activities. In microorganisms only partial correlation with ammonification is observed, although microfungi are the dominant group not only in ammonification (Köhler, Kunze, 1979) but also in respiration (Anderson, Domsch, 1975). The correlation between thiobacilli and

Table 3. Partial correlations (+) positive and (−) negative (0 = no correlation)

	SO_4^{2-}	$H^+_{H_2O}$	H^+_{KCl}	Moisture	C_{ox}	N_{total}	C:N	NH_4^+—N	NO_3^-—N	Aerobic bacteria	Ammonifying bacteria	Thiobacilli	Microfungi	Respiration	Ammonification	Nitrification	Oxidation of $S_2O_3^{2-}$
SO_4^{2-}		+	−	0	−	+	+	0	0								
$H^+_{H_2O}$	+		+	0	+	0	0	0	0								
H^+_{KCl}	−	+			0	0			+					+		+	−
Aerobic bacteria	−	0	0	0	0	−		0	0		+	+	0	0	−		
Ammonifying bacteria	+	−	−	0	+	+		−	−	+		−	0	0	+		
Thiobacilli	+	−	+	+	+	+		0	−	+	−		+	0	0		
Microfungi	0	+	−	0	−	0		0	0	0	0	−		0	0		
Respiration	+	−	0	+	+	0			0	0	0		0				
Ammonification	−	+	0	0	−	+	+	0		−	+		+	+			
Nitrification	+	0	+	0		0		0	0					−	+		
Oxidation of $S_2O_3^{2-}$	−	+	−	+	−	−		−	+	−	+	+	−	+			
Oxidation of S°	0	0	0	+	0		0	0	0	0	0	0	0	0			

respiration, or C_{ox}, may be so understood that the CO_2 production provides chemolithotrophs with an assimilable carbon source.

A positive correlation was found between the concentrations of H^+ and C_{ox} and NO_3^-—N, negative between H^+ and N_{total}. The soil is acidified by organic acids and CO_2 or CO_3^{2-}/HCO_3^- from the carbon cycle and by nitrate from the nitrogen cycle. In non-polluted regions this acidification is more serious than the effect of acid precipitations (Fring, Voigt, 1976). However, action in the reverse direction should also be taken into account: a reduced amount of bacteria with inhibited respiration is not able to utilize available carbon from litter and from root exudates although root secretion is probably reduced (Přikryl, Vančura, 1980) owing to the lower increment rate (Vinš, Pollanschütz, 1977). This may result in a relative cumulation of carbonaceous substances. In addition, the simulation of the immission effect in the experiment inhibited the activity of asymbiotic nitrogen-fixing bacteria. This is valid for Beijerinckia indica (Wodzinski et al., 1978) and the more so to clostridia whose optimum pH is always on the alkaline side. In this way the C:N ratio increases, having its adverse implications for microbial biocenosis. An attempt at representing this assumed effect is shown in Chart B in Fig. 3.

In the polluted plot V, a unit amount of organic matter is colonized by less than

half the number of bacteria, as compared with plot II. In relation to the unit amount of substrate a decrease is observed in respiration and particularly in ammonification (Langkramer, Lettl, 1981, 1982). In this plot No.V the content of NH_4^+—N is extraordinarily high, ammonification being severely inhibited, obviously due to the effect of immissions. However, this ion provides no increase in the extent of microbial biocenosis; in other words, the biological immobilization of

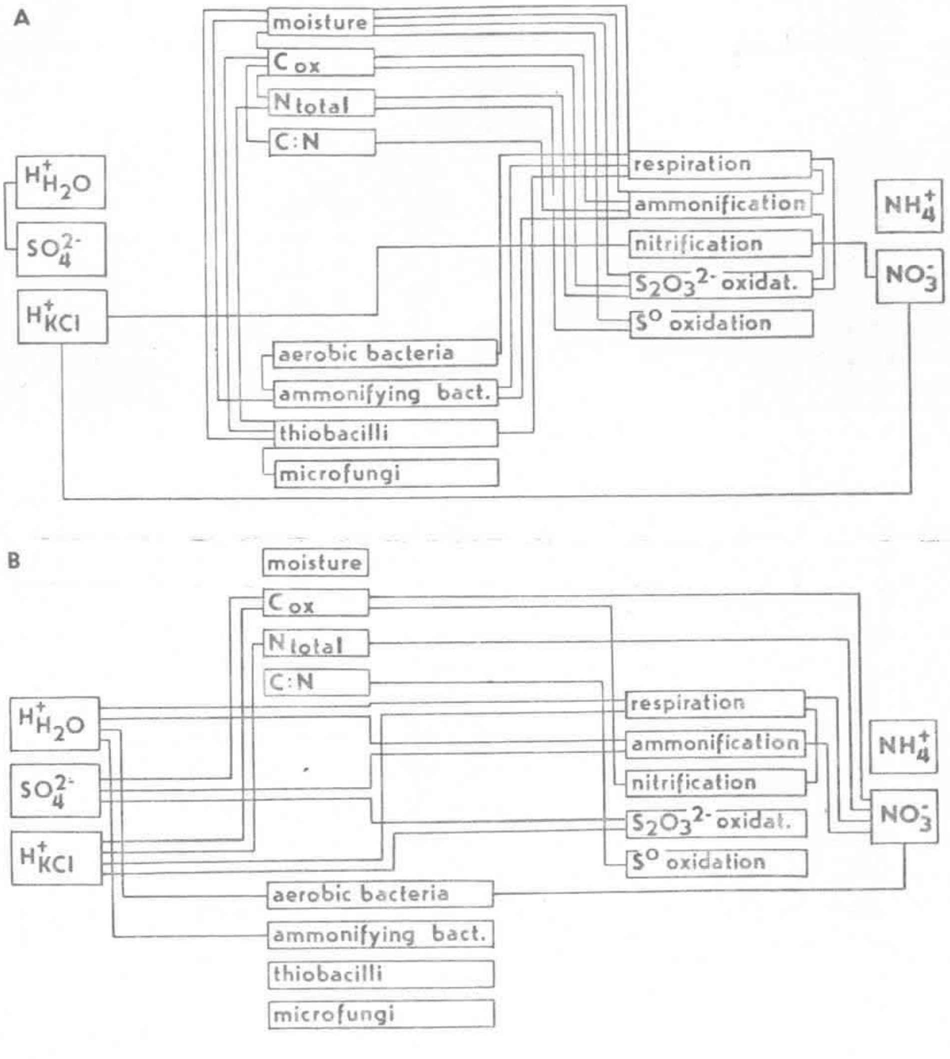

Fig. 1. Simple correlations (A) positive and (B) negative.

nitrogen slows down. Hence microorganisms live in a relative nutrient abundance but they are not able to utilize them. It is to be considered in this connection that chemical intoxication of the soil milieu with SO_2 may play its role here. This idea is justified by the negative correlation among the content of sulphate and bacteria and their activities.

Gaseous SO_2 is sorbed by the soil, particularly at a higher soil moisture

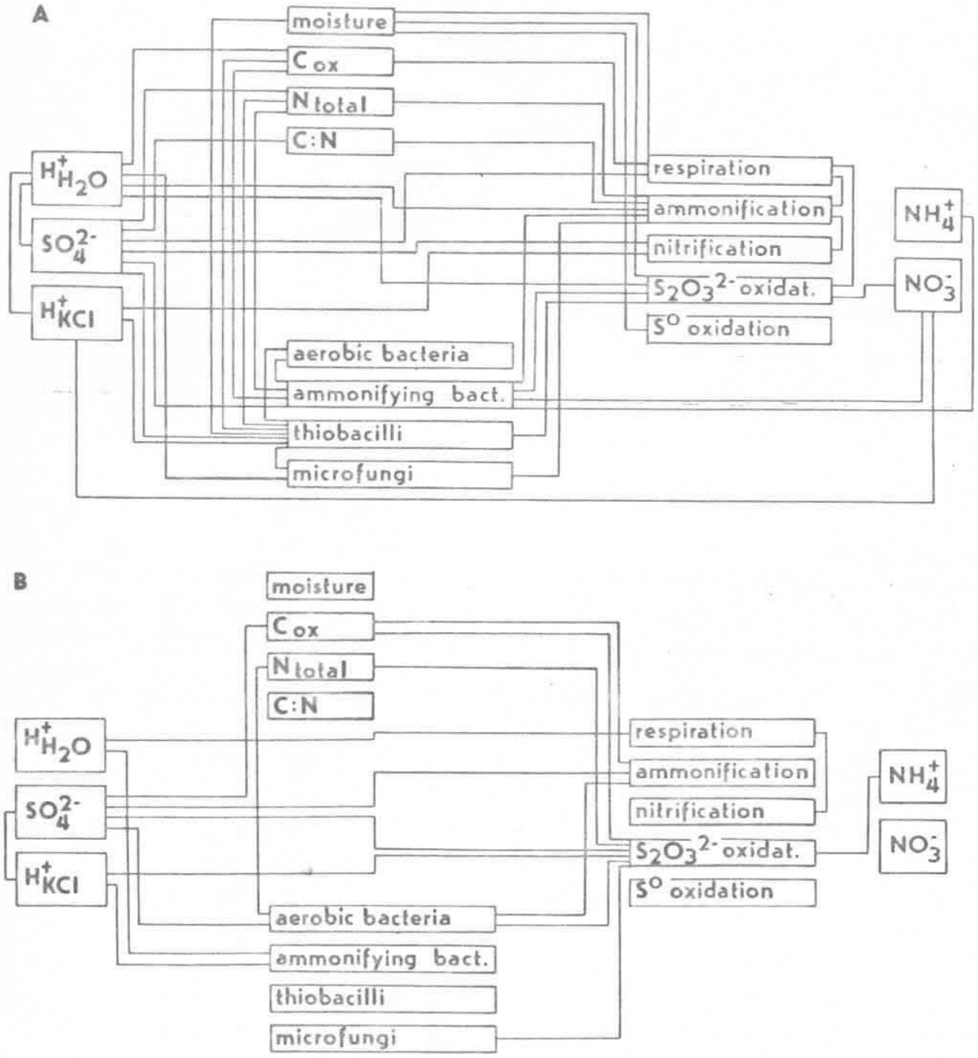

Fig. 2. Partial correlations (A) positive and (B) negative.

(Materna, Kohout, 1980); its high toxicity was clearly demonstrated (Couey, Uota, 1961; Couey, 1965). Its solubilization in aqueous medium produces SO_3^{2-} or HSO_3^- (Vass, Ingram, 1949) — these anions reduce the concentration of bacteria, respiration (Grant et al., 1979a), CO_2 assimilation (Hill, 1971), nitrogen fixation (Wodzinski et al., 1978). They react with membrane lipids and proteins, thus breaking the structural and — thereby — also functional integrity of cells (Lüttge et

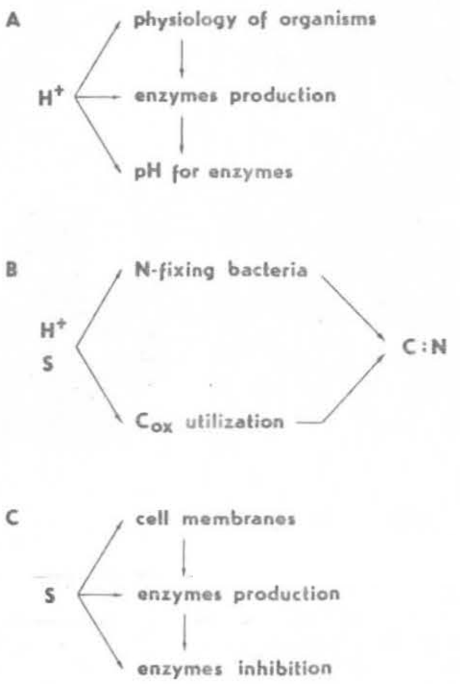

Fig. 3. Proposed mechanisms of negative effects of SO_2 pollution on soil microbial biocenosis.

al., 1972). In a number of enzymes they act as inhibitors (Lyric, Suzuki, 1970; Ziegler, 1974). Sulphates are accompanied in the soil by thiosulphate and tetrathionate (Wainwright, 1978a) which are also toxic (Audus, Quastel, 1947).

In opposition to the manifestation of the toxicity of sulphur compounds is the fact that unrealistically high concentrations (of order of tenths to millimoles of SO_3^{2-}/HSO_3^-) were always needed for obtaining the effect experimentally. The supply of sulphur in severely polluted plot IV was 113 kg/ha wet deposits and 31 kg/ha sulphur directly absorbed in the year 1976 (Materna, Kohout, 1980). There is no probability at all that even such a high sulphur immission level would reach toxic concentrations. In addition to this, SO_3^{2-} is quickly oxidized in the soil to SO_4^{2-} mostly in an abiotic way and only residual amounts can persist for a short time (Ghiorse, Alexander, 1976; Lettl, 1982). Thiosulphate and tetrathionate, the intermediate products of sulphur cycle present in the soils (Wainwright, 1978a), were toxic only under aseptic conditions, not in the soil (Audus, Quastel, 1947).

On the other hand, evidence of the toxic action of sulphur compounds can be seen in the finding that SO_2 penetrates up to several cm into the soil (Materna, Kohout, 1980). In this experiment the sorption of SO_2 by the soil did not take place as a momentary action but its course was stepwise. The presence of SO_2 in soil should be assumed to be variable but permanent under field conditions. An aqueous solution of SO_2 is more toxic than an equivalent amount of HSO_3^- (Grant et al., 1979a). The toxicity of SO_3^{2-}/HSO_3^- is pH-dependent and manifests itself in the acid zone (Labeda, Alexander, 1978; Wodzinski et al., 1978; Babich, Stotzky, 1978; Grant et al., 1979a). Moreover, it markedly increases with the synergic action of NO_2 (Grant et al., 1979b). A number of potentially important side reactions of sulphur is neglected, although at least 19 products were identified (Esmen, Fergus, 1977).

Even despite these contradictions the effect of immissions is at least partly explained by the accumulation of sulphur compounds (Dodd, Lauenroth, 1981) since the effect of pH itself does not suffice to give satisfactory explanation (Wodzinski, Alexander, 1978; Grant et al., 1979a). In our material this seems to hold mainly for ammonification. This activity is able to function within a wide range of conditions with respect to the heterogeneity of the causative organisms but in plots affected by SO_2 this activity is most severely inhibited. Although the activity is negatively correlated with SO_4^{2-} content the immission effect cannot be simulated by means of sulphate (Lettl, 1981). Hence the accompanying sulphur compounds or penetrating SO_2 not included in our set of data are probably involved. The same is likely to hold for the oxidation of $S_2O_3^{2-}$.

The assumed mechanism of action is represented by Chart 3 in Fig. 3.

Although the changes in soil milieu caused by SO_2 immissions (McFee, 1980; Lochman, 1981) are left aside, other factors, hard to determine in this study, may also exert their effect from a purely microbiological point of view. Changes in the structure of the soil microbial biocenosis alone may result in a reduction of mineralizing processes (Odén, 1968). Here importance is attached to the slow-down in cellulose decomposition (Langkramer, 1975); changes were found in sulphur oxidizers (Adamczyk-Winiarska et al., 1975; Wainwright, 1978b; Lettl et al., 1981), and shifts were demonstrated in the species structure of microfungi (Langkramer, 1975; Langkramer, Lettl, 1982). As recognized, a poorer species structure considerably reduces the metabolic activity of the soil (Salonius, 1981).

Although the series of observations used in this study (Langkramer, Lettl, 1981, 1982) is absolutely unique as to the length of duration and extent of analyses, it is obvious that our efforts have failed to take into account all the factors needed for explaining the effect of immissions on soil microbial biocenosis.

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Lettl A., Langkramer O.: **Vzájemné vztahy v mikrobní biocenose půdy smrkového porostu pod vlivem průmyslových imisí SO_2.**

Výpočtem jednoduchých a parciálních korelačních koeficientů byly studovány vztahy mezi imisními vlivy, obsahem živin, koncentraci mikrobů a jejich aktivitami ve fermentačním horizontu A_{02} pěti smrkových porostů odlišně zatížených spadem SO_2. K vysvětlení negativního vlivu imisí na půdní aktivity byly navrženy mechanismy: a) zvýšená koncentrace H^+ ovlivňuje fyziologii mikrobů, mění jejich produkci enzymů, uvolněné enzymy jsou vzdáleny od optima pH; b) imisní vlivy působí kumulaci uhlíkatých látek, spojenou se změnami poměru C:N; c) toxické sloučeniny síry narušují strukturální a tím i funkční integritu mikrobů, alterují jejich enzymatickou výbavu a inhibují uvolněné enzymy.

Využitá řada pozorování (Langkramer, Lettl, 1982) i přes velký rozsah sledovaných faktorů nedává dostatek podkladů k plnému vysvětlení účinku imisí SO_2 na půdní mikrobní biocenosu.

Леттл А., Лангкрамер О.: **Взаимоотношения в микробном биоценозе почвы еловых насаждений находящихся под влиянием промышленных имиссий SO_2.**

Вычислением простых и парциальных корреляционных коэффициентов были исследованы отношения между имиссионными влияниями, содержанием питательных веществ, концентрацией микробов и их активностями в ферментативном горизонте A_{02} пяти еловых насаждений, которые различно загружены имиссиями SO_2. К обяснению негативного влияния имиссий на почвенную активность были предложены механизмы: а) повышенная концентрация H^+ влияет на физиологии микробов, изменяет их продукцию ферментов, выделенные ферменты удалены от оптимума pH; б) имиссии влияют на кумуляцию углеродных веществ, связаную с изменением отношения C:N; в) токсические соединения серы нарушают структуральную и функциональную интегрированность микробов, изменяют их ферментативное оснощение и ингибируют выделенные ферменты.

Использованный ряд исследований (Лангкрамер, Леттл, 1982) и все это большое количество исследованных факторов не дает достаток оснований к полному обяснению влияния имиссий SO_2 на почвенный микробный биоценоз.